Essential Oils from Wild Populations of Algerian *Lavandula stoechas* L.: Composition, Chemical Variability, and *in vitro* Biological Properties

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In an effort to develop local productions of aromatic and medicinal plants, a comprehensive assessment of the composition and biological activities of the essential oils (EOs) extracted from the aerial flowering parts of wild growing *Lavandula stoechas* L. collected from eleven different locations in northern Algeria was performed. The oils were characterized by GC-FID and GC/MS analyses, and 121 compounds were identified, accounting for 69.88–91.2% of the total oil compositions. The eleven oils greatly differed in their compositions, since only 66 compounds were common to all oils. Major EO components were fenchone (**2**; 11.27–37.48%), camphor (**3**, 1.94–21.8%), 1,8-cineole (**1**; 0.16–8.71%), and viridiflorol (**10**; 2.89–7.38%). The assessed *in vitro* biological properties demonstrated that the DPPH-based radical-scavenging activities and the inhibition of the β-carotene/linoleic acid-based lipid oxidation differed by an eight-fold factor between the most and the least active oils and were linked to different sets of molecules in the different EOs. The eleven EOs exhibited good antimicrobial activities against most of the 16 tested strains of bacteria, filamentous fungi, and yeasts, with minimum inhibitory concentrations (*MIC*s) ranging from 0.16 to 11.90 mg/ml.

1. Introduction. – The genus *Lavandula* L. includes 39 species, numerous hybrids, and nearly 400 registered cultivars [1]. Its natural distribution area stretches from the Canary Islands, Cape Verde Islands, and Madeira, across the Mediterranean basin, the Arabian Peninsula, and all the way to tropical North Africa, and, with a disjunction, to India [2]. This genus is represented in the *Algerian Flora* by six species, *i.e.*, *L. stoechas* L., *L. multifida* L., *L. coronopifolia* Poir., *L. pubescens* Dec., *L. dentata* L., and the more recently added *L. antineae* Maire [1–3].

In Algeria, *L. stoechas* L. (syn. *Stoechas officinarium* Moench) is known as '*Helhal*' and is widely distributed across all the northern fringes of the country. It has been reported that this plant is widespread throughout the Mediterranean basin, where it can be a common component of low-growing shrub vegetations on acidic soils (heath) [1]. The large distribution of *L. stoechas* in many countries bordering the Mediterranean

Sea is fairly unique within the genus *Lavandula*. The reported medicinal properties of this species are also very diverse, since *L. stoechas* has traditionally been used as expectorant, carminative, stimulant, wound healing adjuvant [4][5], antispasmodic, sedative, diuretic, therapy for rheumatic diseases [6], analgesic, and antiseptic [7]. Most of these properties are due to the essential oil (EO) fraction of the plant. In the Algerian folk medicine, the aerial parts of *L. stoechas*, especially the inflorescences, are used as an antiseptic and stimulant agent [8], and in the Algerian cuisine, they are also used as culinary herb to prepare the most popular couscous.

The composition of *L. stoechas* EO has been studied from wild and cultivated plants collected in several Mediterranean countries. About 60 chemical constituents have been described. These mostly include mono- and sesquiterpenes. The most commonly reported chemotype is a fenchone/camphor chemotype, though a fenchone/1,8-cineole and a pulegone chemotype have also been reported [4–6][9–20]. Additional compounds have been detected in non-EO extracts of *L. stoechas*. These include flavonoids [1], longipinene derivatives [21], and triterpenoids [22].

Several recent studies have revealed that *L. stoechas* EO and other extracts have antimicrobial [4][6][17][19][23], insecticidal [24], and antioxidant [7] properties. Nevertheless, most of those studies only assayed single EO samples, so that the chemical basis for the biological properties of *L. stoechas* EO could not be assessed. The antioxidant properties were also very scantly characterized.

As part of our ongoing efforts to characterize and valorize the Algerian aromatic and medicinal plants, we conducted an exhaustive investigation of the chemical composition and variability of EOs obtained from the flowering aerial parts of *L. stoechas* harvested in eleven localities in Algeria (*Fig. 1* and *Table 1*). We also conducted the first characterization of the DPPH radical-scavenging and β-carotene/linoleic acid-bleaching properties of *L. stoechas* EO and tried to correlate these activities with the abundance of specific compounds in the EO. Antimicrobial properties of the EOs were also assessed.

2. Results and Discussion. – 2.1. *Extraction Yields.* Conventional hydrodistillation of the aerial flowering parts of the eleven wild populations of *L. stoechas* from northern Algeria yielded light to dark yellow EOs with yields ranging from 0.34 to 1.63% (*w*/*w*, on the dry weight basis; *Table 1*). Even for the four populations harvested at the same locality, *i.e.*, *LS4*, *LS5*, *LS6*, and *LS7* (*Fig. 1*), the EO yields exhibited large variability, spreading from 0.52 to 1.63%. This may be a typical feature of wild *L. stoechas*, since wild Spanish populations of *L. stoechas* also exhibited similarly high variations of EO yields [10]. Similar conclusions were also drawn for wild populations from Crete [12] and Corsica [13]. These differences in EO yields may be the result of either uncontrolled biotic and abiotic elicitations or of inherent genetic differences, as already described for other EO producing plant species [25][26].

2.2. *Essential Oil Composition and Variability.* The EOs of eleven *L. stoechas* populations originating from different locations of northern Algeria (*Fig. 1* and *Table 1*) were subjected to detailed GC-FID and GC/MS analyses, to improve the current knowledge about the *L. stoechas* EO composition and its possible variability. The identified constituents are listed in *Table 2*, according to their elution order on a *HP-1* capillary column. Their retention indices (*RI*s) on *HP-1* and *INNOWAX-1*

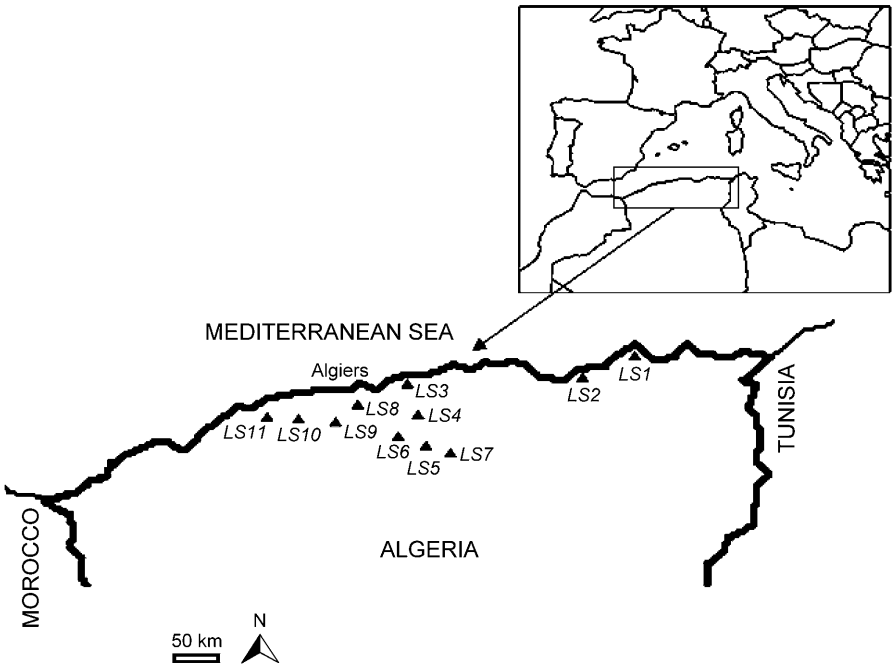

Fig. 1. *Geographical origins of the eleven Algerian populations of* L. stoechas *used in this study.* For the significance of the population codes, see *Table 1.*

Table 1. *Collection Sites, Main Ecological Features, and Essential Oil* (EO) *Yields of the Eleven Algerian* L. stoechas *Populations that Served as a Source of EOs in this Study*

Population code	Locality	Collection site	Bioclimatic zone[a])	Rainfall [mm/year]	Geographical location			EO Yield [%, *w*/*w*]
					Latitude N	Longitude E	Elevation [m]	
LS1	Skikada	Aïn-Chéraïa	MH	905	36°51′21″	6°43′12″	478	0.71
LS2	Jijel	Irageune	MH	1200	36°49′22″	5°45′45″	66	0.79
LS3	Boumerdes	Boumerdes	MH	900	36°45′49″	3°28′59″	88	1.16
LS4	Bouira	Lakhdaria	CH	660	36°34′12″	3°34′11″	128	0.52
LS5	Bouira	Ain Bessam	CH	660	36°17′53″	3°39′38″	685	1.63
LS6	Bouira	Guerrouma	CH	660	36°23′44″	3°26′23″	481	1.14
LS7	Bouira	Taguedit	CSA	400	36°09′01″	3°41′21″	200	0.60
LS8	Blida	Hammam Melouane	MH	600	36°28′16″	2°49′46″	346	1.36
LS9	Médéa	Benchicao	CH	615	36°11′53″	2°50′55″	1122	0.34
LS10	Ain Defla	Ain Defla	MSA	470	36°09′14″	2°03′59″	383	0.36
LS11	Chlef	Chlef	MSH	420	36°09′54″	1°19′25″	162	0.52

[a]) Bioclimatic zones: MH, Mediterranean humid; CH, continental humid; CSA, continental semi-arid; MSA, Mediterranean semi-arid; MSH, Mediterranean sub-humid.

Table 2. *Chemical Composition of the Essential Oils from the Flowering Aerial Parts of Eleven Algerian Wild Populations of* L. stoechas

Components[a])	$RI_{HP\text{-}1}$[b])	RI_{INNO}[c])	Composition (%)											Identification[d])
			LS1[e])	*LS2*	*LS3*	*LS4*	*LS5*	*LS6*	*LS7*	*LS8*	*LS9*	*LS10*	*LS11*	
Benzaldehyde[f])	929	1546	–[g])	–	–	–	–	–	–	tr[h])	–	–	–	GC, MS
α-Pinene	932	1037	–	–	–	–	–	–	–	0.06	–	–	–	GC, MS, CoGC
α-Fenchene	943	1077	–	–	–	–	–	–	–	0.03	–	–	–	GC, MS
Camphene[i])	945	1088	tr	–	–	–	–	–	–	0.11	–	–	–	GC, MS
Verbenene[i])	948	1147	–	–	–	–	–	–	–	tr	–	–	–	GC, MS, CoGC
Hexanoic acid[f])[i])	949	1860	0.37	–	0.11	0.29	–	–	–	–	0.3	0.19	0.13	GC, MS
Oct-1-en-3-ol[i])	959	1463	0.07	–	–	0.07	–	–	–	0.02	0.14	0.07	–	GC, MS
β-Pinene	972	1129	–	–	–	–	–	–	–	0.03	–	–	–	GC, MS, CoGC
2,3-Dehydro-1,8-cineole[f])[i])	979	1211	–	–	–	–	–	–	–	0.04	–	–	–	GC, MS
Lavander lactone[f])[i])	998	1700	0.21	0.1	0.13	0.06	0.08	0.26	0.17	0.06	0.16	0.12	0.11	GC, MS
α-Phellandrene	999	1185	–	–	–	–	–	–	–	0.01	–	–	–	GC, MS
Benzyl alcohol[f])[i])	1004	1881	0.19	–	0.09	0.07	–	–	0.15	0.04	0.24	0.11	0.05	GC, MS
δ-3-Carene	1007	1173	–	–	–	–	–	–	–	0.12	–	–	–	GC, MS, CoGC
o-Cymene[i])	1010	1287	–	0.05	0.2	0.12	0.03	–	0.11	0.06	–	0.19	0.22	GC, MS
α-Terpinene	1011	1202	–	0.07	0.2	0.12	0.11	–	0.09	0.03	–	0.12	0.02	GC, MS, CoGC
p-Cymene	1013	1295	–	0.08	tr	tr	0.1	–	0.44	0.22	–	–	–	GC, MS, CoGC
Limonene[i])	1023	1219	–	–	–	–	–	tr	–	–	–	–	–	GC, MS, CoGC
1,8-Cineol (1)	1023	1229	**0.16**	**8.71**	**8.49**	**4.28**	**4.19**	**2.21**	**1.3**	**7.51**	**2.63**	**5.69**	**7.85**	GC, MS, CoGC
o-Cresol[f])[i])	1034	–	–	–	–	–	–	–	–	0.03	0.07	0.05	–	MS
p-Cresol[f])[i])	1046	2062	0.08	tr	tr	0.05	–	–	0.05	0.07	0.09	–	–	GC, MS
γ-Terpinene	1050	1269	tr	tr	tr	tr	tr	tr	tr	0.03	tr	tr	tr	MS
trans-Thujan-4-ol[i])	1054	1482	0.05	–	–	–	0.11	0.12	0.07	0.11	tr	0.06	0.11	GC, MS
cis-Linalool oxide (furanoid)[i])	1058	1463	0.26	0.41	0.21	0.5	0.25	1.15	0.37	0.39	0.45	0.45	0.38	GC, MS
Camphenilone[f])[i])	1060	1486	0.12	0.06	tr	0.05	tr	tr	0.11	0.02	0.04	0.05	0.07	GC, MS
Fenchone (2)	1068	1427	**11.27**	**37.48**	**24.11**	**24.14**	**36.76**	**16.35**	**14.56**	**34.48**	**26.52**	**29.92**	**32.42**	GC, MS, CoGC
p-α-Dimethylstyrene[f])[i])	1073	1463	–	–	–	–	–	–	–	0.09	–	–	–	GC, MS
trans-Linalool oxide (furanoid)[i])	1073	1491	0.34	0.4	0.22	0.39	0.29	1.01	0.29	0.33	0.38	0.35	0.35	GC, MS
Terpinolene	1080	1308	tr	tr	tr	tr	tr	tr	tr	0.01	tr	tr	tr	MS
Nonanal[f])[i])	1082	1414	0.02	–	–	–	–	–	–	–	0.05	–	tr	MS
Linalool	1084	1561	0.36	0.48	0.15	0.5	0.23	1.06	0.45	0.15	0.46	0.37	0.53	GC, MS, CoGC
cis-Thujan-4-ol[i])	1084	1569	0.06	–	–	–	0.1	–	–	0.11	0.07	0.1	0.1	GC, MS

Table 2 (cont.)

Components[a])	$RI_{HP\text{-}1}$[b])	RI_{INNO}[c])	Composition (%)											Identification[d])
			LS1[e])	*LS2*	*LS3*	*LS4*	*LS5*	*LS6*	*LS7*	*LS8*	*LS9*	*LS10*	*LS11*	
Hotrienol[f])[i])	1084	1627	0.12	0.07	tr	–	0.03	tr	tr	0.01	tr	0.1	tr	GC, MS
2-Phenylethyl alcohol[f])[i])	1084	1936	0.12	tr	0.43	0.1	0.09	0.13	0.24	0.04	0.05	0.12	0.27	GC, MS, CoGC
α-Thujone[f])[i])	1087	1463	0.08	0.16	0.19	0.15	0.14	0.14	0.25	0.08	0.29	0.2	0.14	GC, MS
α-Fenchol	1099	1607	0.42	1.37	0.61	0.75	1.01	0.58	0.93	1.0	1.3	0.79	0.8	GC, MS, CoGC
Chrysanthenone[i])	1100	1505	0.1	0.13	0.16	0.22	0.06	0.01	0.65	0.13	tr	0.09	0.09	GC, MS
cis-p-Mentha-2,8-dien-1-ol[i])	1104	1649	0.05	0.11	0.06	tr	0.08	–	–	0.08	–	–	–	GC, MS
α-Campholenal[i])	1105	1510	0.16	0.22	0.24	0.17	0.29	0.13	0.27	0.15	0.21	0.23	0.19	GC, MS
cis-Pinan-2-ol[f])[i])	1107	–	0.05	0.19	0.19	0.11	–	0.16	0.11	0.15	0.15	0.23	0.22	MS
Nopinone[f])[i])	1108	1616	tr	0.12	0.01	0.07	–	0.05	0.1	0.19	0.1	0.08	0.14	GC, MS
Camphor (3)	1121	1556	**9.77**	**5.81**	**1.94**	**17.12**	**15.50**	**21.8**	**11.56**	**8.69**	**8.99**	**13.19**	**14.52**	GC, MS, CoGC
trans-Pinocarveol[i])	1124	1683	0.42	0.37	1.61	0.24	0.22	0.22	0.94	0.24	0.33	0.37	0.29	GC, MS
cis-Verbenol[i])	1125	1675	0.45	0.56	0.45	0.3	0.22	0.34	0.36	0.28	0.46	0.54	0.48	GC, MS
Sabina ketone[f])[i])	1125	1668	0.04	0.07	0.12	tr	0.03	0.01	0.05	0.06	0.06	0.06	0.11	GC, MS
***trans*-Verbenol**[i])	1127	1699	**3.0**	**2.33**	**0.11**	**1.0**	**0.83**	**0.76**	**0.7**	**0.03**	**1.29**	**1.33**	**0.58**	GC, MS
α-Phellandren-8-ol[f])[i])	1130	1727	tr	0.18	tr	0.13	tr	–	–	tr	tr	tr	0.13	GC, MS
exo-Methylcamphenilol[f])[i])	1133	1625	0.14	0.09	0.21	0.1	0.18	0.09	0.35	0.1	0.14	0.1	0.1	GC, MS
Pinocarvone[i])	1139	1604	0.19	0.31	0.14	0.3	0.13	0.13	0.25	0.3	0.17	0.19	0.26	GC, MS
β-Phellandren-8-ol[f])[i])	1145	1735	0.16	0.15	0.02	0.52	0.19	0.15	0.25	0.32	0.18	0.16	tr	GC, MS
δ-Terpineol[f])[i])	1146	1699	0.05	0.5	0.64	0.16	0.13	0.76	0.45	0.26	0.34	0.57	0.88	GC, MS, CoGC
cis-Linalool oxide (pyranoid)[f])[i])	1148	1762	0.51	0.18	tr	0.18	0.1	0.29	0.15	0.17	0.29	0.42	0.16	GC, MS
Borneol	1150	1720	1.13	0.2	0.21	0.71	0.73	0.52	0.95	0.32	0.63	0.87	0.29	GC, MS, CoGC
Lavandulol	1150	1688	0.20	0.11	0.21	0.09	0.08	0.11	0.13	0.11	0.3	0.29	0.18	GC, MS, CoGC
trans-Linalool oxide (pyranoid)[f])[i])	1152	1783	0.42	0.21	tr	0.18	0.15	0.2	0.28	0.16	0.35	0.37	0.23	GC, MS
p-Methylacetophenone (**4**)[f])[i])	1154	1803	0.18	0.16	0.28	0.11	0.11	0.29	0.4	0.18	0.18	0.22	0.18	GC, MS
***m*-Cymen-8-ol (5)**[f])[i])	1155	1867	**0.14**	**0.23**	**2.06**	**2.02**	**1.7**	**0.42**	**0.42**	**3.21**	**0.79**	**0.11**	**0.81**	GC, MS
***p*-Cymen-8-ol**	1160	1874	**2.64**	**1.32**	**2.0**	**1.81**	**1.60**	**1.31**	**1.78**	**2.77**	**2.74**	**1.53**	**1.83**	GC, MS
Terpinen-4-ol	1162	1622	**0.32**	**0.5**	**1.17**	**0.31**	**0.18**	**0.3**	**2.14**	**0.4**	**0.35**	**0.35**	**0.5**	GC, MS, CoGC
Myrtenal[i])	1170	1665	0.43	0.5	0.48	0.36	0.33	0.27	0.43	0.36	0.56	0.49	0.47	GC, MS
α-Terpineol	1172	1715	0.22	0.55	0.62	0.21	0.3	0.41	0.37	0.41	0.53	0.39	0.96	GC, MS, CoGC
Methylchavicol[f])[i])	1175	1705	–	0.26	0.64	0.16	0.17	–	0.28	0.09	0.05	0.23	0.15	GC, MS
Myrtenol	1179	1821	0.59	0.66	0.56	0.41	0.3	0.47	0.66	0.56	1.13	0.8	0.64	GC, MS

Table 2 (cont.)

Components[a])	$RI_{HP\text{-}1}$[b])	RI_{INNO}[c])	Composition (%)											Identification[d])
			LS1[e])	*LS2*	*LS3*	*LS4*	*LS5*	*LS6*	*LS7*	*LS8*	*LS9*	*LS10*	*LS11*	
Verbenone	1180	1749	**2.67**	**2.18**	**1.22**	**1.78**	**1.43**	**0.7**	**1.36**	**2.07**	**1.49**	**1.22**	**0.9**	GC, MS
trans-Piperitol[f])[i])	1191	1775	–	–	–	0.15	–	–	–	–	–	–	–	GC, MS
trans-Carveol[i])	1197	1860	0.88	0.81	0.69	0.42	0.59	0.48	0.71	0.4	0.86	0.57	0.54	GC, MS
exo-2-Hydroxycineole[f])[i])	1198	1881	0.18	tr	0.22	0.09	0.03	0.04	0.35	0.14	0.55	0.49	0.34	GC, MS
α-Fenchyl acetate[i])	1207	1495	tr	0.37	0.12	0.12	0.15	0.2	0.28	0.36	0.21	0.31	0.25	GC, MS
Cuminaldehyde[i])	1213	1821	tr	0.06	0.15	0.26	0.13	tr	0.08	0.08	0.23	0.05	0.28	GC, MS
Carvone	1215	1769	0.52	0.74	0.83	0.34	0.52	0.46	0.71	0.58	0.71	0.67	0.79	GC, MS, CoGC
cis-Piperitone oxide[f])[i])	1222	–	0.74	0.09	0.48	0.29	0.29	0.13	0.58	0.45	0.59	0.31	0.15	GC, MS
2-Phenylethyl acetate[f])	1225	1839	0.08	–	0.4	tr	tr	–	0.08	0.12	0.29	0.12	0.17	GC, MS, CoGC
trans-2-Hydroxypinocamphone[f])[i])	1226	–	0.18	–	–	–	0.06	–	–	–	–	–	–	MS
Piperitenone[i])	1226	1764	0.11	–	–	0.08	0.18	–	0.19	–	–	–	–	GC, MS
trans-Piperitone oxide[f])[i])	1232	–	0.85	0.26	tr	0.1	0.33	0.3	0.32	0.11	0.3	0.22	0.1	GC, MS
Geraniol[f])[i])	1233	1866	0.14	0.09	–	0.23	–	–	0.29	0.17	0.21	0.12	0.28	GC, MS, CoGC
Perillaldehyde[f])[i])	1245	1829	0.18	0.13	0.17	0.13	0.14	0.21	1.31	0.12	0.27	0.26	0.18	GC, MS
Cuminol[f])[i])	1263	2134	–	–	–	–	–	–	–	0.09	–	0.25	0.18	GC, MS
Thymol[i])	1267	2213	tr	–	–	0.09	–	–	–	tr	tr	tr	tr	GC, MS, CoGC
α-Necrodyl acetate (**6**)[i])	1269	1601	–	–	0.83	–	–	–	1.34	–	–	–	–	GC, MS
Bornyl acetate	1270	1612	**2.82**	**0.47**	**0.46**	**2.36**	**3.03**	**3.73**	**4.0**	**1.61**	**1.52**	**3.12**	**0.92**	GC, MS, CoGC
Lavandulyl acetate	1271	1606	0.13	tr	tr	tr	0.05	tr	tr	0.08	0.11	0.11	0.08	GC, MS, CoGC
Undecan-2-one[f])[i])	1273	1619	–	–	0.61	0.4	tr	–	–	–	–	–	–	GC, MS
Carvacrol[i])	1276	2240	tr	–	–	0.24	–	–	–	0.02	tr	tr	tr	GC, MS, CoGC
Unknowns (MM150 and MM164)	1276	–	0.46	–	2.03	1.6	2.18	0.58	0.51	2.39	1.32	0.27	1.25	MS
Filifolide A[f])[i])	1278	–	0.58	0.14	tr	0.12	tr	–	–	0.06	0.17	0.11	tr	MS
Myrtenyl acetate[i])	1305	1704	**1.27**	**0.61**	**2.17**	**1.69**	**1.62**	**2.54**	**1.72**	**1.01**	**2.51**	**1.68**	**2.14**	GC, MS, CoGC
Piperitenone[i])	1308	1967	0.14	tr	tr	0.08	tr	tr	tr	0.03	0.09	tr	tr	GC, MS
Eugenol[f])[i])	1327	2196	0.20	0.08	0.13	0.19	–	0.08	0.44	0.04	0.29	0.23	0.08	GC, MS, CoGC
Unknown (MM168)	1351	2157	1.02	–	–	0.21	0.36	0.44	0.29	0.13	0.19	0.21	0.14	MS
Cyclosativene[i])	1369	1506	0.06	0.11	0.1	0.24	0.07	–	–	0.11	–	0.16	0.07	GC, MS
α-Copaene	1377	1517	tr	tr	tr	0.06	–	–	–	0.06	–	0.08	0.06	GC, MS, CoGC
Sativene[f])[i])	1391	1569	–	–	–	–	–	–	–	0.02	–	–	–	GC, MS
trans-β-Caryophyllene[i])	1419	1628	–	–	–	–	–	–	–	0.08	–	–	–	GC, MS, CoGC

Table 2 (cont.)

Components[a])	$RI_{HP\text{-}1}$[b])	RI_{INNO}[c])	Composition (%)											Identification[d])
			LS1[e])	*LS2*	*LS3*	*LS4*	*LS5*	*LS6*	*LS7*	*LS8*	*LS9*	*LS10*	*LS11*	
Alloaromadendrene[i])	1459	1681	–	–	–	–	–	–	–	0.09	–	–	–	GC, MS
β-Selinene[f])[i])	1482	1750	0.21	0.11	–	0.05	–	–	0.11	0.05	–	0.13	–	GC, MS
Dihydroactinidiolide[f])[i])	1486	–	0.17	–	–	–	–	–	–	–	–	–	–	MS
Epicubebol (**7**)[f])[i])	1488	1967	0.57	0.52	0.15	0.47	0.17	0.33	0.14	0.26	0.34	1.0	0.52	GC, MS
Ledene[i])	1491	1730	0.16	0.15	–	0.36	–	–	–	0.09	0.28	0.25	0.14	GC, MS
α-Muurolene	1494	1753	0.16	0.25	–	0.19	–	0.13	0.14	0.10	0.17	0.13	0.08	GC, MS, CoGC
γ-Cadinene	1508	1792	0.05	0.03	0.12	0.06	tr	0.01	0.02	0.04	0.1	0.26	0.06	GC, MS
Cubebol (**8**)[f])[i])	1509	1914	0.38	0.71	0.44	0.25	0.16	0.29	0.32	0.29	0.38	0.15	0.67	GC, MS
Calamenene[i])	1510	1860	0.15	tr	0.21	0.2	tr	0.12	0.1	0.12	0.08	0.21	0.11	GC, MS
δ-Amorphene (**9**)[f])[i])	1511	1730	0.23	0.59	0.18	0.46	tr	0.17	0.22	0.15	0.2	0.59	0.32	GC, MS
δ-Cadinene	1515	1787	0.11	tr	tr	tr	tr	tr	tr	0.04	0.04	0.12	0.06	GC, MS
Cadina-1,4-diene[f])[i])	1525	1816	0.18	tr	–	0.14	–	–	–	0.06	–	0.12	–	GC, MS
α-Calacorene[f])[i])	1529	1946	–	tr	–	0.18	–	0.16	–	0.12	0.11	0.2	–	GC, MS
Myrtenyl valerate[f])[i])	1542	–	0.69	0.56	0.33	0.26	0.22	0.45	0.24	0.1	0.14	0.41	0.2	GC, MS
cis-α-Copaen-8-ol[f])[i])	1550	2077	0.54	0.57	0.7	0.28	0.39	1.24	–	0.38	0.3	0.45	1.06	GC, MS
Palustrol[f])[i])	1562	1973	0.27	tr	0.17	0.15	tr	0.21	0.15	0.11	tr	0.14	tr	GC, MS
Spathulenol	1565	2157	0.22	tr	0.23	0.19	tr	0.31	0.27	0.08	0.27	0.16	0.28	GC, MS
Caryophyllene oxide	1571	2024	0.81	0.55	0.98	0.31	0.53	2.22	0.6	0.52	0.48	0.65	0.46	GC, MS
Globulol	1576	2095	0.82	0.98	0.59	0.2	0.28	0.67	0.52	0.45	0.57	0.73	0.38	GC, MS
Viridiflorol (**10**)[i])	1583	2103	**7.38**	**4.88**	**6.81**	**4.34**	**3.58**	**7.14**	**6.44**	**3.01**	**3.8**	**5.07**	**2.89**	GC, MS
Ledol[i])	1593	2065	**1.01**	**1.70**	**1.96**	**2.09**	**0.5**	**1.8**	**1.96**	**0.47**	**1.28**	**1.24**	**0.99**	GC, MS
Unknown	1594	–	3.27	1.23	2.09	0.56	1.57	4.48	4.09	1.36	1.22	1.77	0.86	
Epicubenol[f])	1616	2095	0.60	0.64	0.62	0.51	0.27	0.79	0.59	0.37	0.61	0.63	0.4	GC, MS
α-Cadinol[i])	1626	2203	0.43	0.36	0.7	0.55	0.41	0.92	0.51	0.3	0.52	0.5	0.38	GC, MS
α-Muurolol[f])[i])	1630	2233	0.38	0.2	0.25	tr	tr	tr	0.31	0.08	tr	–	–	GC, MS
Cubenol[f])	1633	2101	0.22	0.22	0.06	0.1	–	–	0.07	0.06	–	0.09	–	GC, MS
β-Eudesmol[f])[i])	1636	2268	0.43	0.31	0.35	0.16	–	–	0.11	0.09	–	–	–	GC, MS
O-Containing sesquiterpene (MM218)	1654	–	1.67	1.66	1.37	1.11	0.51	1.31	1.01	0.7	0.98	1.09	0.56	MS
α-Cyperone[f])[i])	1725	2398	0.39	–	–	–	–	–	–	–	–	–	–	GC, MS

Table 2 (cont.)

Components[a])	$RI_{HP\text{-}1}$[b])	RI_{INNO}[c])	Composition (%)											Identification[d])
			LS1[e])	*LS2*	*LS3*	*LS4*	*LS5*	*LS6*	*LS7*	*LS8*	*LS9*	*LS10*	*LS11*	
O-Containing sesquiterpene (MM236)	1822	–	2.43	2.15	2.46	2.17	1.15	4.77	2.22	1.48	3.75	2.55	2.78	MS
O-Containing sesquiterpene (MM236)	1829	–	1.83	1.73	1.98	1.75	0.92	3.83	1.98	1.2	3.05	1.99	2.11	MS
Monoterpene hydrocarbons			0.0	0.2	0.4	0.24	0.24	0.0	0.64	0.71	0.0	0.31	0.24	
O-Containing monoterpenes			46.19	71.3	55.27	66.27	75.37	61.32	55.73	71.33	62.36	70.87	74.43	
Sesquiterpene hydrocarbons			1.31	1.24	0.61	1.94	0.07	0.59	0.59	1.13	0.98	2.25	0.9	
O-Containing sesquiterpenes			20.38	17.18	19.82	14.63	8.87	25.83	17.2	9.85	16.33	16.44	13.48	
Others			2.0	0.55	2.41	1.78	0.29	0.21	1.24	0.63	1.74	1.33	0.85	
Total identified			*69.88*	*90.47*	*78.51*	*84.86*	*84.84*	*87.95*	*75.4*	*83.65*	*81.41*	*91.2*	*89.9*	

[a]) Components listed in the order of elution on the *HP-1* capillary column. [b]) *RI:* Retention indices on the *HP-1* column. [c]) *RI:* Retention indices on the *INNOWAX-1* column. [d]) Identification: GC, identification based on *RI*; MS, identification based on a comparison of mass spectra with *Wiley* and *Nist* libraries and the literature [27][28]; Co-GC, identification based on co-injection with authentic compounds. [e]) For the signification of the population code, see *Table 1* and *Fig. 1*. [f]) Components reported for the first time in *L. stoechas* oils. [g]) –: Not detected. [h]) tr: Trace ($< 0.01\%$). [i]) Components reported for the first time in *L. stoechas* oils from Algeria.

capillary columns, their relative percentages (FID-based quantification), and their methods of identification are also listed in *Table 2*. The analyses resulted in the identification of 121 compounds that represented 69.88–91.2% of the total oil compositions. To the best of our knowledge, 83 of these compounds are being reported here for the first time in Algerian *L. stoechas* oil, and 54 compounds had never been reported in *L. stoechas* oils (*Table 2*). These include *p*-methylacetophenone (**4**), *m*-cymen-8-ol (**5**), α-necrodyl acetate (**6**), epicubebol (**7**), cubebol (**8**), and δ-amorphene (**9**; *Table 2* and *Fig. 2*). In total, 66 constituents were detected in all populations, while the others could only be seen in one or more populations.

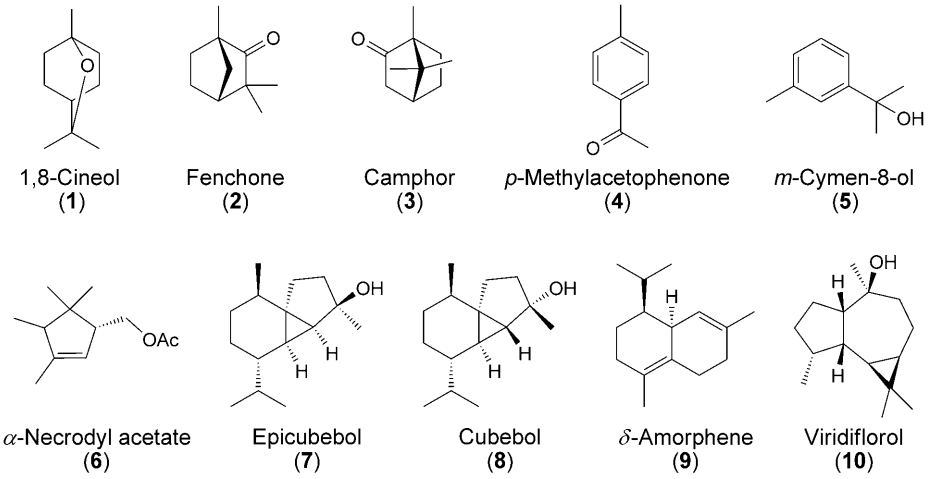

Fig. 2. *Structures of the main compounds* (**1**–**3** and **10**) *of the Algerian* L. stoechas *essential oils and some compounds* (**4**–**9**) *detected for the first time in essential oils of* L. stoechas

The most abundant compound class was constituted of the O-atom containing monoterpenes (46.19–75.37%), with fenchone (**2**; 11.27–37.48%), camphor (**3**; 1.94–21.8%), and 1,8-cineol (**1**; 0.16–8.71) as the most abundant compounds (*Table 2* and *Fig. 2*). The O-atom containing sesquiterpenes composed the second most abundant compound class (8.87–25.83%), with viridiflorol (**10**; 2.89–7.38) as the most abundant representative (*Fig. 2*). The EOs were, however, poor in sesquiterpene hydrocarbons (0.07–2.25%) and monoterpene hydrocarbons (0.0–0.71%).

To characterize the chemical basis of the EO composition variability of wild Algerian *L. stoechas* populations, we conducted a hierarchical clustering analysis (HCA) on an EO composition data matrix restricted to the four major components, *i.e.*, **1**–**3** and **10**, which accounted for 28.58 to 60.03% of the total EO contents. This separated the Algerian *L. stoechas* populations into two main categories (*Fig. 3*). The first one (*Cluster I*) contained the EOs of all populations, except *Population LS6*. These ten EOs had **2** as major component, whereas the EO of *Population LS6* had **3** as principal compound. Linkage cluster analysis of *Cluster I* revealed the existence of a large chemical diversity among the fenchone chemotype populations, with the separation of *Subcluster IA*, including the populations *LS1* and *LS7*, from the other populations of *Cluster I* on the basis of higher contents of **10** (7.38 and 6.44%, resp.) and

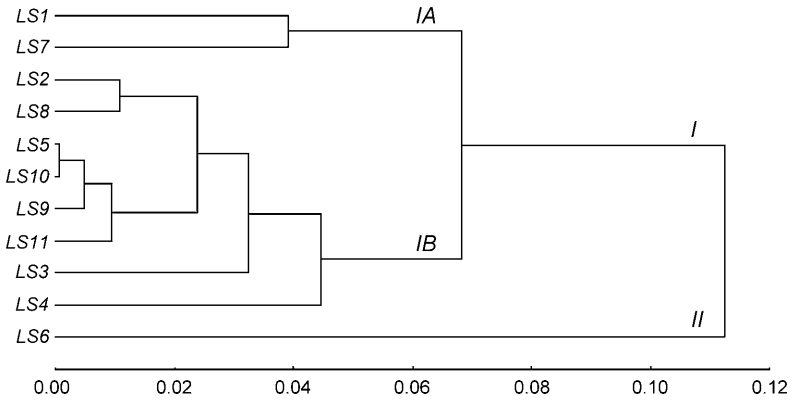

Fig. 3. *Hierarchical cluster analysis dendrogram of the eleven Algerian populations of* L. stoechas*, based on a comparison of the contents of the four major essential oil constituents.* For the significance of the population codes, see *Table 1*.

lower contents of **1** (0.16 and 1.30%, resp.). Subsequent subgrouping dichotomically separated the other EOs on the basis of their different proportions of **1**, **3**, and **10**.

The most reported chemotype of *L. stoechas* is a fenchone/camphor chemotype recorded in Morocco [16], Tunisia [19], Spain [10], Italy [6], Crete, Greece [12], Hatay, Turkey [17], Cyprus [11], and Corsica, France [14]. In agreement with the present data, comparative studies of different populations from a restricted geographic area have revealed that the ratio between compounds **2** and **3** may vary greatly and may even be inverted [10–14][16]. In a few of these studies conducted in Spain and Crete, some populations with a fenchone/1,8-cineol chemotype were distinguished, that were growing in the same phytogeographic area as others with a fenchone/camphor chemotype [10][12]. In Turkey, a 1,8-cineol/camphor chemotype was described [18]. In the EOs of the Algerian populations *LS2* and *LS3*, **1** was the second most abundant component behind **2**, and it represented more than 8% of the total oil content in these populations. Nevertheless, statistical analyses of our denser sampling does not support the distinction of a fenchone/camphor from a fenchone/1,8-cineol chemotype in Algeria. It appears from the present data, that populations with high contents of **1** are not restricted to the northern shores of the Mediterranean Sea, but can also be found in northern Africa.

The eleven Algerian *L. stoechas* EOs studied differed greatly from other published *L. stoechas* EOs with respect to less abundant compounds. *p*-Cymene was revealed as a fairly abundant constituent (6.5% of the total oil content) in the EO of another Algerian population [20], in contrast to the EOs of the present study, where it was, at best, detected at trace amounts (*Table 2*). Inversely, the same study on an Algerian population [20] did not detect any myrtenyl acetate, which was an important constituent of the present oils (0.61–2.54%) and a characteristic component of other *L. stoechas* EOs [10]. The composition of the EOs reported here differed from any other by the presence of higher quantities of **10** (2.89–7.38%) and the absence of linalyl acetate, β-phellandrene, longifolene, and germacrene D.

2.3. *Antioxidant Activity.* EOs with antioxidant activity are of interest because of their lengthened shelf life and as source of natural antioxidants. Despite the wide use of *L. stoechas* EO, its antioxidant potential has never been assessed. Determined by the DPPH-based free radical-scavenging assay, the eleven Algerian *L. stoechas* EOs exhibited significant differences in their radical-scavenging activity (*Table 3*). The EO of *Population LS4* exhibited the highest radical-scavenging activity, followed by the *LS2* oil, with $EC_{50(DPPH)}$ values of 5.10 ± 0.32 and 10.97 ± 0.98 mg/ml, respectively. The *LS5* and *LS7* oils were the least active with respective $EC_{50(DPPH)}$ values of 32.42 ± 1.46 and 26.80 ± 0.91 mg/ml. Hence, the $EC_{50(DPPH)}$ values of the most and least active oils differed by an eight-fold factor. When compared to the reference compounds butylhydroxytoluene (BHT) and α-tocopherol, all *L. stoechas* oils were found to be less efficient radical scavengers than α-tocopherol and BHT, which exhibited $EC_{50(DPPH)}$ values of 0.02 ± 0.001 and 0.11 ± 0.01 mg/ml, respectively (*Table 3*).

Table 3. *DPPH Free Radical-Scavenging and β-Carotene/Linoleic Acid-Bleaching Activities of the Essential Oils of Algerian* L. stoechas *Populations in Comparison with BHT and α-Tocopherol*

Sample	$EC_{50(DPPH)}$ [mg/ml][a])	AA [%][b])
Essential oils[c])		
LS1	24.73 ± 0.73	25.54 ± 0.36
LS2	10.97 ± 0.98	68.79 ± 0.62
LS3	15.50 ± 0.57	16.32 ± 0.46
LS4	5.10 ± 0.32	79.72 ± 0.45
LS5	32.42 ± 1.46	21.60 ± 1.20
LS6	19.38 ± 0.36	26.71 ± 0.07
LS7	26.80 ± 0.91	28.87 ± 0.52
LS8	13.75 ± 0.72	50.85 ± 1.10
LS9	12.26 ± 0.32	42.12 ± 0.41
LS10	16.44 ± 1.49	28.47 ± 1.12
LS11	20.70 ± 0.99	26.46 ± 0.72
Positive controls		
BHT	0.11 ± 0.01	99.86 ± 0.19
α-Tocopherol	0.02 ± 0.001	98.40 ± 0.38

[a]) $EC_{50(DPPH)}$: Concentration of EO that scavenged 50% of the DPPH radicals. Lower EC_{50} values indicate higher reactivity with DPPH and, hence, a better H-atom donating activity. Values are means of three parallel determinations of three independent experiments ± standard deviations. [d]) AA: Potential of EO to inhibit the oxidation of linoleic acid. Higher AA values indicate greater inhibition of the linoleic acid oxidation. Values are means of three parallel determinations of three independent experiments ± standard deviations. [c]) For the signification of the population codes, see *Table 1* and *Fig. 1*.

With a complementary assay, the β-carotene/linoleic acid-bleaching test, the potential of the EOs to inhibit the oxidation of linoleic acid, expressed as AA (antioxidant activity [%]), was determined. This test is known to reveal different antioxidant properties of natural products than the DPPH test and may thus unveil different antioxidant compounds within plant extracts [29]. Among the eleven *L. stoechas* oils, the highest activity was again associated with *Population LS4* (AA = $79.72 \pm 0.45\%$), followed by *Population LS2* (AA = $68.79 \pm 0.62\%$), as can be seen in

Table 3. All oils were, nevertheless, less potent inhibitors of linoleic acid oxidation than the reference compounds BHT and α-tocopherol (AA = 99.86 ± 0.19 and 98.40 ± 0.38%, resp.).

Several compounds that we found to be present in *L. stoechas* EOs are known to possess antioxidant activities. These include eugenol, carvacrol, thymol, terpinolene, α-terpinene, γ-terpinene [30], and terpinen-4-ol [31]. To determine whether some EO components play an important role in the antioxidant activity of Algerian *L. stoechas* EO, we calculated the *Pearson*'s correlation coefficient between the contents of the 93 most abundant EO components and the DPPH- and β-carotene/linoleic acid-based antioxidant activities of the eleven oils. No significant correlation was found between the contents of the four major EO components (**1**–**3** and **10**) and these two activities. This agrees with the TLC screening test (data not shown) and with previous reports [30]. Only for three compounds that were present in at least seven EOs, positive correlations with $r^2 > 0.4$ with the β-carotene/linoleic acid test were obtained, *i.e.*, for pinocarvone ($r^2 = 0.583$, $p = 0.006$), β-phelandren-8-ol ($r^2 = 0.511$, $p = 0.013$), and α-muurolene ($r^2 = 0.506$, $p = 0.014$), and only for one compound with the DPPH test, *i.e.*, ledene ($r^2 = 0.445$, $p = 0.025$). Unfortunately, their non-availability under a pure form did not allow experimental confirmation of these correlations. Nevertheless, additional compounds may participate in the antioxidant activity of some of the eleven EOs. The very active *LS4* oil was, for example, the only oil to contain thymol and carvacrol, two compounds with known antioxidant activity. These data suggest that the antioxidant activity of different Algerian *L. stoechas* EOs may be due to different constituents. This conclusion is confirmed by a rapid TLC screening test of the components of the eleven oils. The most active EO, that of *Population LS4*, displayed the largest number of clearance spots (≥ 6 spots in total, which included carvacrol that was tested in parallel as a standard), while the least active oils generated less clearance spots (data not shown).

2.4. *Antimicrobial Activity.* Because of the increased resistance of many pathogenic microbes to drugs, additional efforts are required to unveil novel compounds from natural sources, including plants and their EOs. Therefore, *in vitro* bioassays to estimate the susceptibility of sixteen pathogenic and phytopathogenic microorganisms towards the EOs of the eleven Algerian *L. stoechas* populations were conducted (*Table 4*). In agreement with the high variability of their chemical compositions, the eleven EOs were variably effective against the tested microorganisms. The minimum inhibitory concentrations (*MIC*s) varied between 0.23 and 11.36 mg/ml against the bacteria, between 0.23 and 8.52 mg/ml against the filamentous fungi, and between 0.16 and 11.9 mg/ml against the yeast species (*Table 4*).

An antimicrobial activity is considered strong, when the *MIC* values range between 0.05 and 0.50 mg/ml, moderate when they are between 0.6 and 1.50 mg/ml, and weak when they are above 1.50 mg/ml [32]. Accordingly, the EO of *Population LS3* can be considered as very active against all tested microbial strains. The EO of *Population LS6* exhibited the same pattern of activity except against *A. flavus*, *F. oxysporum*, and *C. albicans* (b), against which it was moderately active. The *LS5* EO exhibited moderate antimicrobial activity towards all bacteria and filamentous fungal strains with maximum *MIC*s of 1.4 mg/ml. It showed, however, only a weak activity against the *C. albicans* strains.

Table 4. *Minimum Inhibitory Concentrations* (*MIC*) *of the Essential Oils of Algerian* L. stoechas *Populations*

Microorganism	*MIC* [mg/ml]										
	LS1[a])	*LS2*	*LS3*	*LS4*	*LS5*	*LS6*	*LS7*	*LS8*	*LS9*	*LS10*	*LS11*
Gram-negative bacteria											
E. coli E13	4.84	3.84	0.23	2.04	1.4	0.32	4.76	11.36	3.78	3.7	1.96
E. coli E195	6.05	>7.68	0.23	>8.16	0.7	0.32	1.19	8.52	3.78	5.18	1.96
E. coli ATCC 25925	1.21	1.28	0.23	0.51	0.7	0.32	1.19	8.52	2.16	1.48	1.96
E. coli K12	4.84	3.84	0.23	2.55	0.7	0.32	1.19	11.36	2.16	2.96	0.49
K. pneumoniae E40	4.84	>7.68	0.23	>8.16	0.7	0.32	1.19	8.52	2.7	0.74	0.49
K. pneumoniae Kp19	4.84	>7.68	0.23	>8.16	0.7	0.32	1.19	11.36	2.7	3.7	1.96
S. enterica	4.84	>7.68	0.23	>8.16	0.7	0.32	1.19	11.36	2.7	3.7	1.96
P. aeruginosa	1.21	>7.68	0.23	>8.16	0.7	0.32	1.19	8.52	2.16	4.44	1.96
A. baumannii	4.84	>7.68	0.23	5.1	0.7	0.32	1.19	8.52	2.16	0.74	0.98
Gram-positive bacteria											
B. subtilis	4.84	3.84	0.23	2.55	0.7	0.32	1.19	11.36	2.16	1.48	0.98
Filamentous fungi											
A. niger	6.05	4.48	0.46	2.55	0.7	0.32	5.95	8.52	2.7	5.18	1.96
A. flavus	6.05	5.12	0.46	2.55	1.4	0.64	5.95	8.52	2.7	5.18	1.96
F. oxysporum f. sp. *lini*	4.84	2.56	0.23	2.04	1.4	0.64	5.95	8.52	2.16	3.7	0.98
M. ramannianus	4.84	2.56	0.23	2.04	1.4	0.32	1.19	11.36	2.16	1.48	0.98
Yeasts											
C. albicans (a)	6.05	4.48	0.46	2.55	3.5	0.16	5.95	8.52	10.80	3.7	1.96
C. albicans (b)	6.05	>6.4	0.46	>8.16	2.8	0.80	11.9	11.36	10.80	5.18	1.96

[a]) For the signification of the population codes, see *Table 1* and *Fig. 1*.

The antimicrobial activities of plant EOs have been reported to be linked to the presence of specific constituents. Several studies have reported that camphor, linalool, 1,8-cineole [33], carvacrol, eugenol, perillaldehyde [34], terpinen-4-ol [35], caryophyllene oxide, spathulenol [36], borneol [37], and myrtenal [6] revealed powerful antimicrobial activities. All of these compounds were present in our oils, some being even major oil constituents. Nevertheless, our data (statistical analysis) did reveal that no significant correlation between the content of a specific EO component and the antimicrobial activity of the EO did exist, against any of the tested microorganisms (data not shown). This may be due to the fact that several of the major compounds share a similar antimicrobial activity. Additionally, synergistic effects may have occurred, as known for compounds **1** and **3** [38].

The present results on the antimicrobial activities of *L. stoechas* EOs agree with those in previous reports, indicating, for example, that the volatile oil of wild Tunisian plants exhibited antibacterial activity against *Staphylococcus aureus* [19]. Similarly, antibacterial activities were reported for EOs of wild *L. stoechas* populations from Turkey [4][17] and Cagliari, Italy [6], and for cultivated plants in Australia [23]. The EOs of *L. pedunculata*, formerly considered as a subspecies of *L. stoechas*, have shown a significant antifungal activity against dermatophyte strains [39].

3. Conclusions. – The EOs extracted from eleven wild populations of Algerian *L. stoechas* displayed a large diversity in their composition that affected major and minor constituents. The gained knowledge about their composition further expands the known and already impressive range of diversity of volatile organic compounds in this species, with the first detection of many minor constituents. With its large chemical biodiversity, *L. stoechas* was confirmed to be a very good source of plant material for a large range of bioactive molecules. Nevertheless, the study also confirmed that variations in the contents of major *L. stoechas* EO components were not restricted to (and may not match) known evolutionary events, such as radiation or sub-speciation events, although the origin of radiation, diversification, and current location for subspecies biodiversity has clearly been assigned in Spain [1]. A future gain of knowledge on the molecular events behind the biosynthesis of individual EO components will now open a fascinating area of research to unveil the genetic and environmental events that generate such biodiversity.

The data reported in this study suggest, for the first time, that the antioxidant activity of different *L. stoechas* EOs is moderate and can be due to the presence of different sets of antioxidant compounds. Because our sampling was made on a small geographic area, this analysis again highlights the extraordinary biodiversity of *L. stoechas* in terms of source material for bioactive compounds.

The antimicrobial activity of Algerian *L. stoechas* EO is reported here for the first time. Our analyses validate the traditional use of the EO of this species as an antiseptic drug in Algeria and in some Mediterranean regions. Nevertheless, some populations (*LS3* and *LS6*) proved to be a better source of bioactive material against a wider panel of microorganisms than the others and may therefore have a greater applicative potential in the pharmaceutical, crop protection, and cosmetic industries.

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Experimental Part

Plant Material. The aerial parts of *L. stoechas* L. (flowering shoots with leaves and inflorescences) were collected at maximum flowering (March 2007) from wild-growing populations. A total of eleven populations were sampled randomly across Algerian northern parts (*Fig. 1* and *Table 1*). The plant material was harvested at the same period (March 2007) to avoid potential seasonal variations in the EO composition. Botanical authentication of the species was carried out at the herbarium of the Department of Botany, Ecole Nationale d'Agronomie (ENA), Algiers, Algeria, where voucher specimens have been deposited, and according to the *Flora of Algeria* [3]. The harvested material was shade air-dried and finely powdered using a blender before hydrodistillation.

Isolation of Essential Oils. The powdered flowering aerial parts were subjected to hydrodistillation for 3 h using a modified *Clevenger*-type apparatus. The EOs were isolated from the distillates by the addition of Et_2O and dried (anh. Na_2SO_4). After filtration, the Et_2O was completely evaporated under vacuum. The pure oils were weighed and stored at 4° in brown glass vials prior to analysis. The oil yields are compiled in *Table 1*. The oil color ranged from light to dark yellow.

GC Analysis. The GC-FID analyses were carried out with an *Agilent 6890* apparatus equipped with two cap. columns of different polarity, a *HP-1* (50 m × 0.32 mm i.d., film thickness 0.52 μm) and an *INNOWAX-1* cap. column (60 m × 0.32 mm i.d., film thickness 0.50 μm). The oven temp. was programmed from 80 to 295° at 2°/min and held isothermal at 295° for 25 min for the *HP-1* column

and from 60 to 245° at 2°/min and held isothermal at 245° for 25 min for the *INNOWAX-1* column. He was used as the carrier gas at a flow rate of 1.5 and 1.3 ml/min for the *HP-1* and *INNOWAX-1* column, resp. Aliquots of 1 µl of the samples diluted in hexane were injected in the split mode (ratio 1:20). The injector and flame ionization detector (FID) temp. was 250°.

GC/MS Analysis. The GC/MS analyses of the EOs were carried out with an *Agilent HP* gas chromatograph (model *6850* and *7890*) coupled to an *Agilent HP* mass selective detector (model *5975* and *5975C*, resp.) in the electron impact mode (EI) equipped with Chem-Station data analysis software for mass spectrum database screening. In total, 0.2 µl aliquots of the samples diluted in hexane were injected using the splitless mode. The same *INNOWAX-1* column as described above and a *HP-1* cap. column (50 m × 0.20 mm i.d., film thickness 0.33 µm) were used. The oven temp. was programmed from 60 to 280° at 3°/min and held isothermal at 280° for 10 min for the *HP-1* column and from 60 to 245° at 3°/min and held isothermal at 245° for 25 min for the *INNOWAX-1* column; injector temp., 250°; carrier gas, He (1.3 ml/min). The EI-MS operating parameters were as follows: electron energy, 70 eV; automatic scanning of the mass range 30–350 amu (30–400 amu for the *INNOWAX-1* column) at 2.4 scans/s; ion source temp., 230°; quadrupole, 150°.

Compound Identification. Retention indices (*RI*s) were calculated for all EO constituents rel. to the t_R of *n*-alkanes that were analyzed under the same chromatographic conditions [40]. The identification of the EO constituents was based on the comparison of the mass spectra with those of the *WILEY275.L* and *NIST05.L* libraries (computer matching), those of a homemade mass spectral library, those of authentic compounds available in our laboratory, and published mass spectra [27]. The identification was confirmed by comparing the *RI*s with those of authentic compounds and previously published *RI*s [27][28]. Relative percentage amounts of the separated compounds were calculated from the GC-FID peak areas generated by a computerized integrator without the use of correction factors. The reported values are the means obtained from three injections of each oil sample.

Rapid TLC Screening Test. As described by *Cuendet et al.* [41], 5 µl aliquots of the EOs diluted 1:10 in hexane, of BHT, and of α-tocopherol were spotted on silica gel (SiO_2) sheets and developed in acetone/hexane 1:30 (*v*/*v*). The TLC sheets were sprayed with a 0.2% soln. of DPPH (=1,1-diphenyl-2-picrylhydrazyl) reagent in EtOH and left in the dark at r.t. for 30 min. The spots for which the color turned from purple to yellow on a violet background were scored as positive radical-scavenging compounds.

DPPH Radical-Scavenging Method. This is the most widely used method for evaluating the radical-scavenging activity of plant drugs. The test assesses the capacity of the oil to scavenge the relatively stable DPPH radical. Scavenging of the DPPH free radical causes a change of color from an initially dark violet soln. to a yellow one, due to the formation of DPPH-H (=1,1-diphenyl-2-picrylhydrazine). According to *Brand-Williams et al.* [42], aliquots of 50 µl of solns. of various concentrations of each oil (2–44 mg/ml) and of each standard (0.005–16 mg/ml) were added to 5 ml of an EtOH soln. of freshly prepared DPPH (0.004%), and the mixtures were vortexed and incubated in the dark at r.t. for 30 min. The disappearance of the DPPH radical was read spectrophotometrically at 517 nm against a blank (EtOH soln.) with a *JASCO-V530* spectrophotometer. The percentage of DPPH free radicals scavenged (I [%]) was calculated using *Eqn. 1:*

$$I\,[\%] = (A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}} \times 100 \quad (1)$$

where A_{blank} is the absorbance of the control reaction containing all reagents except the EOs and A_{sample} is the absorbance of the test reaction containing also the oil. The $EC_{50(\text{DPPH})}$ value represented the concentration of EO that scavenged 50% of the DPPH radicals and was used as an estimate of the radical-scavenging activity. It was calculated from the plot of *I* [%] against the EO concentrations. All tests were carried out in triplicate and the $EC_{50(\text{DPPH})}$ values were reported as means ± SD.

β-Carotene/Linoleic Acid-Bleaching Method. This is one of the complementary methods used to screen for antioxidant compounds. It is based on the principle that the unsaturated fatty acid linoleic acid spontaneously oxidizes when it is exposed to the reactive oxygen species (ROS) present in H_2O enriched with O_2. The reaction products then initiate β-carotene oxidation into a colorless form. The extent of discoloration is measured spectrophotometrically and used as an estimate of the antioxidant activity

(AA). As described in by *Dapkevicius et al.* [43], a stock soln. of β-carotene/linoleic acid emulsion mixture was prepared as follows: 0.5 mg of crystalline β-carotene was dissolved in 1 ml of $CHCl_3$, and 1 ml of this soln. was transferred into a round flask containing 25 µl of linoleic acid and 200 mg of *Tween-40*. After the complete removal of $CHCl_3$ by evaporation, 100 ml of O_2 enriched dist. H_2O was added under vigorous shaking. Aliquots (2.5 ml) of the mixture were transferred into a series of test tubes containing 350 µl of EO diluted in EtOH (2 g/l). After vigorous mixing, the test tubes were incubated at 50° for 2 h. A negative control tube was prepared by replacing the EO by EtOH. The absorbance was finally measured at 490 nm against a blank (emulsion without β-carotene). Antioxidant activities (*AA* [%]) were calculated using *Eqn. 2:*

$$AA\ [\%] = (1 - (A_0 - A_t)/(A_{0\text{control}} - A_{t\text{control}})) \times 100 \quad (2)$$

where A_0 and $A_{0\text{control}}$ were the absorbance at t_0 of the sample and the control, resp., and A_t and $A_{t\text{control}}$ were the absorbance at $t = 2$ h of the sample and the control, resp. All tests were carried out in triplicate and the inhibition percentages (*AA* [%]) were reported as means ± SD.

Microbial Strains. A selection of pathogenic and phytopathogenic microorganisms including ten bacteria, four filamentous fungi, and two yeasts were used to evaluate the antimicrobial activity of *L. stoechas* EOs. These included the *Gram*-negative bacteria *Escherichia coli* (E13), *E. coli* (E195), *E. coli* (ATCC 25925), *E. coli* (K12), *Klebsiella pneumoniae* (E40), *K. pneumoniae* (Kp19), *Salmonella enterica* (E32), *Acinetobacter baumannii* (E16), and *Pseudomonas aeruginosa* (CIP A22), the *Gram*-positive bacterium *Bacillus subtilis* (ATCC 6633), the filamentous fungi *Aspergillus niger*, *A. flavus*, *Fusarium oxysporum* f. sp. *lini* (Foln 3–5), and *Mucor ramannianus* (NRRL 1829), and the yeasts *Candida albicans* (strains a and b). All pure cultures of the microorganisms were obtained from the collection of the Laboratory of Microbiology, Ecole Normale Supérieure, Kouba-Alger, Algeria, except for the yeast strains that were clinically isolated from human patients at the *Zmirli* hospital, Alger, Algeria.

Antimicrobial Activity. The minimum inhibitory concentrations (*MIC*s) for the test microorganisms were determined *in vitro* by using the conventional agar dilution method, as previously described [44]. Serial dilutions of the EOs were made with sterile melted nutrient agar medium enriched with 10 g/l glucose and *Tween-80* (0.5%, *v*/*v*) to cover a concentration range of 0.16–24 mg/ml. After vortexing, the resulting nutrient agar solns. were immediately poured into *Petri* dishes. The dishes were allowed to dry at r.t. and were spot-inoculated in two replicates with 2 µl of each target microorganism suspension. Inocula of the microorganisms were prepared from 24 h cultures for bacteria and 72 h cultures for fungi, and the suspensions were adjusted to *ca.* 10^6 CFU/ml. The same test was carried out without EO as a control. The inoculated plates were incubated at 30° for 24 h for bacteria and for 48 h for fungi. The *MIC* values were the lowest concentrations of each EO that yielded no visible growth of the microorganisms.

Statistical Analyses. The cluster analysis (*Euclidean* distance and 1-*r Pearson* method) was performed with STATISTICA version 5.1 software (*Stat Soft, Inc.*, Tulsa, OK, USA) on the quant. data (GC-FID analyses) of the four main EO constituents of the eleven EO samples, to evaluate their respective degrees of relatedness. To identify EO compounds potentially associated with biological activities, *Pearson*'s correlation coefficients were calculated between the EO contents of the compounds and the biological activities. To facilitate the interpretation of the results for the DPPH radical-scavenging activity, the $EC_{50(\text{DPPH})}$ values were transformed into $100 - EC_{50(\text{DPPH})}$, to have values that would increase with an increasing radical-scavenging power. The significance of the correlation by pairs of components was tested using *Bonferroni*'s correction for multiple comparisons. The level of significance was set at $\alpha = 0.05$, and a p value < 0.0005 was accepted as significant.

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